Figure 11 is the amino acid sequence of human claudin-8 wherein the extracelluar domains are underlined.

Figure 12 is the amino acid sequence of human claudin-9 wherein the extracelluar domains are underlined.

Figure 13 is the amino acid sequence of human claudin-10 wherein the extracelluar domains are underlined.

Figure 14 is the amino acid sequence of human occludin wherein the extracelluar domains are underlined.

DESCRIPTION OF THE INVENTION

The instant invention satisfies the foregoing needs and fulfills additional objects and advantages by providing novel pharmaceutical compositions that include a biologically active agent and a permeabilizing agent effective to enhance mucosal delivery of the biologically active agent in a mammalian subject. The permeabilizing agent reversibly enhances mucosal epithelial paracellular transport, typically by modulating epithelial junctional structure and/or physiology at a mucosal epithelial surface in the subject. This effect typically involves inhibition by the permeabilizing agent of homotypic or heterotypic binding between epithelial membrane adhesive proteins of neighboring epithelial cells. Target proteins for this blockade of homotypic or heterotypic binding can be selected from various related junctional adhesion molecules (JAMs), occludins, or claudins.

Epithelial cells provide a crucial interface between the external environment and mucosal and submucosal tissues and extracellular compartments. One of the most important functions of mucosal epithelial cells is to determine and regulate mucosal permeability. In this context, epithelial cells create selective permeability barriers between different physiological compartments. Selective permeability is the result of regulated transport of molecules through the cytoplasm (the transcellular pathway) and the regulated permeability of the spaces between the cells (the paracellular pathway).

Intercellular junctions between epithelial cells are known to be involved in both the maintenance and regulation of the epithelial barrier function, and cell-cell adhesion. The tight junction (TJ) of epithelial and endothelial cells is a particularly

JAM-1-dependent homophilic adhesion. In particular, JAM-1 may dimerize in cis-interactions yielding parallel homodimers positioned at one cell surface, and the cis-dimerization might expose an interface available for homophilic adhesive interactions between JAM-1 molecules on opposing cell surfaces. This model could account for homotypic adhesion between adjoining cells within confluent endothelial or epithelial monolayers. In addition, JAM-1 dimers expressed on transmigrating leukocytes are proposed to interact with JAM-1 dimers expressed on endothelial cells, thus accounting for the adhesion and de-adhesion events that occur during leukocyte transendothelial migration. (Dejana, et al., Throb. Haemost. 86: 308-315, 2001)

A crystal structure of a recombinant soluble form of murine JAM-1 protein (a truncated extracellular region of the molecule designated "rsJAM") has been described. (Kostrewa, et al., EMBO J., 20: 4391-4398, 2001). The rsJAM construct is proposed to consist of two immunoglobulin-like domains connected by a conformationally restrained short linker. Two JAM molecules reportedly form a U-shaped dimer by complementary interactions including two salt bridges between respective rsJAM constructs. The report further identifies a central tri-peptide of rsJAM (Arg58-Val59-Glu60) that corresponds to a proposed conservative "motif for dimerization". This conservative motif, "R(V,I,L)E", is suggested to mediate formation of rsJAM dimers in solution. The R(V,I,L)E motif, as well as flanking residues Trp61, Lys62, Cys73, and Tyr74, are noted by the authors to be conserved in published sequences of murine, bovine and human JAM-1. Moreover, the sequence R(V,I,L)E is noted to also be conserved in JAM-2 and JAM-3.

Studies of mutant rsJAM that have been engineered to introduce a disruptive point mutation in the proposed dimerization motif (Glu60Arg), suggest that the mutation blocks homotypic aggregation of rsJAM (Kostrewa, et al., EMBO J., 20: 4391-4398, 2001). Based on these mutant studies and on analysis of crystal packing of rsJAM, a more detailed model for homophilic adhesion of JAM has been proposed. In this model, JAM cis-dimers are believed to form on the cell surface, and the cis-dimerization is proposed to be a necessary precursor to adhesive trans-interactions between dimerized JAM molecules on opposing cell surfaces.

Additional studies have reported the identification of human, rat, and bovine counterparts of murine JAM-1 (see, e.g., Liu et al., J. Cell. Sci. 113:2363-2374, 2000; Ozaki et al., J. Immunol. 163:553-557, 1999; Wiliams et al., Mol. Immunol. 36:1175-1188, 1999; and Sobocka et al., Blood 95: 2600-2609, 2000). These different JAM-1

homologues exhibits between 68%-75% overall amino acid identity with the murine JAM-1 protein sequence. There is said to be a "[s]triking sequence similarity in the transmembrane and cytoplasmic tail regions in particular—suggesting an important and conserved function for these proteins perhaps involving protein interactions at the cytoplasmic interface (see, e.g., Williams et al. (Mol. Immunol. 36:1175-1188, 1999). There is also noted to be general structural conservation among these different JAM-1 homologs in terms of their extracellular structure—which each exhibit amino-terminal and carboxy-terminal β-sandwich folds proposed to represent tandem V_H- and C_2-type Ig-like domains (see, e.g., Dejana, et al. (Throb. Haemost. 86: 308-315, 2001).

The putative extracellular domain of human JAM-1 was recently expressed as a fusion protein to generate anti-human JAM-1 antibodies that inhibited transepithelial resistance recovery (TER) in T84 cell monolayers after tight junction disruption mediated by transient calcium depletion (Liu et al., J. Cell. Sci. 113:2363-2374, 2000). In particular, the anti-JAM antibodies inhibit JAM-1 and occludin redistribution to TJs following calcium mediated disruption. However, these authors report that purified recombinant human JAM-1 containing the extracellular domain does not inhibit TER after tight junction disruption, contrary to published results for murine JAM-1. On this basis it is considered that the data may not support a model of extracellular homo- or heterotypic interaction mediated by the human JAM-1 extracellular domain. In another study investigating the structure/function of human JAM-1, Williams et al. (Mol. Immunol. 36:1175-1188, 1999) report that both murine and hunman JAM-1 Fc chimeras and transfected COS cells failed to show homotypic adhesion for the protein *in vitro*—suggesting that "firm adhesion may not be the function of this molecule *in vivo*." In a separate study, Liang et al. (Am. J. Physiol. 279:1733-1743, 2000,) report that a recombinant soluble form of human JAM-1 inhibits recovery of TER following trypsin-EDTA disruption of TJs.

Additional molecules have been identified with apparent homology to JAM-1. A recently identified JAM2 cDNA corresponds to a predicted 34-kD type I integral membrane protein featuring two Ig-like folds and three N-linked glycosylation sites in the extracellular domain. A single protein kinase C phophorylation consensus site and a PDZ-binding motif are predicted in the short cytoplasmic tail. Northern blot analysis suggests that JAM2 is preferentially expressed in the heart (Cunningham et al., J. Biol. Chem., 275: 34750-34756, 2000). In a related International Publication

(WO 01/14404), Cunningham teaches that JAM-2, unlike JAM-1, does not show expression in peripheral blood leukocytes, and that it is unknown whether JAM-2 functions in homotypic interactions. Cunningham speculates that it may be possible to use a fusion between the JAM-2 extracellular sequence and the Fc region of mouse/human IgG to: screen for a JAM-2 ligand; screen for small molecule inhibitor of JAM-2 heterotypic interactions; or to neutralize JAM-2 function in either heterotypic or homotypic interactions.

Another JAM family member, designated "Vascular endothelial junction-associated molecule" (VE-JAM), contains two extracellular immunoglobulin-like domains, a transmembrane domain, and a relatively short cytoplasmic tail. VE-JAM is principally localized to intercellular boundaries of endothelial cells (Palmeri, et al., J. Biol. Chem., 275: 19139-19145, 2000,). VE-JAM is expressed by endothelial cells of venules, and is also expressed by endothelia of other vessels. Another reported JAM family member, JAM-3, has a predicted amino acid sequence that displays 36% and 32% identity, respectively, to JAM-2 and JAM-1. JAM-3 shows widespread tissue expression with higher levels apparent in the kidney, brain, and placenta. At the cellular level, JAM-3 transcript is expressed within endothelial cells. JAM-3 and JAM-2 have been reported to be binding partners. In particular, the JAM-3 ectodomain reportedly binds to JAM2-Fc. JAM-3 protein is up-regulated on peripheral blood lymphocytes following activation. (Pia Arrate, et al., J. Biol. Chem., 276: 45826-45832, 2001).

Another proposed trans-membrane adhesive protein involved in epithelial tight junction regulation is Occludin. Occludin is an approximately 65-kD type II transmembrane protein composed of four transmembrane domains, two extracellular loops, and a large C-terminal cytosolic domain (Furuse et al., J. Cell Biol. 123:1777 - 1788, 1993; Furuse et al., J Cell Biol 127:1617-1626, 1994). This topology has been confirmed by antibody accessibility studies (Van Itallie, and Anderson, J. Cell. Sci. 110: 1113-1121,1997,). The extracellular loops are chemically distinct. The first extracellular loop contains approximately 65% tyrosine and glycine residues. Although the presence of alternating tyrosine and glycine residues is conserved in all five occludin homologs from different animal species presently cloned, the functional significance of this particular sequence is unclear (Fujimoto. J. Cell. Sci. 108:3443 - 3449, 1995).